CHLORELLA SUPPLEMENTATON DECREASES THE BLOOD LEVELS OF DIOXINS IN JAPANESE PREGNANT WOMEN - A PRELIMINARY INVESTIGATION -

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Introduction

Our environments including food have been polluted with extremely toxic dioxins which are polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) [1]. Consequently, humans and breast milk also have already been contaminated with these compounds [2, 3, 4].

Adverse health consequences of dioxins have been investigated in the fetus and suckling which are considered the most sensitive stages of human life. In fact, we have already observed their unfavorable effects on thyroid hormone and immune response systems in Japanese infants perinatally and lactationally exposed to them [5, 6, 7, 8, 9].

In order to avoid or prevent their adverse health consequences on fetuses and sucklings, active reduction of their contamination levels in mother's body, particularly for highly toxic congeners, seems quite important. In rats, dietary fiber and chlorophyll have been shown to promote the fecal excretion of PCDDs and PCDFs probably due to the inhibition of their absorption and reabsorption in the digestive tract to some extent, and therefore to decrease their levels in rat liver [10, 11, 12].

In this study, we examined whether such kinds of effect were observed by *Chlorella* supplements, which was rich with chlorophylls and dietary fibers, for PCDDs, PCDFs and DL-PCBs or not in Japanese pregnant women.

Materials and methods

Chlorella (Parachlorella beijerinckii) is unicellular green algae that grow in fresh water. Compared to other plants, *Chlorella* contains 55~67 % protein, 1~4 % chlorophylls, 9~18 % dietary fibers and large amounts of minerals and vitamins. At present, *Chlorella* is widely sold as a health food and/or health supplement in Japan, the U.S. and other countries.

Subjects of this study comprised 10 healthy pregnant women (age range, 24~39 years) who were receiving prenatal care at Shimomura O.B.G.Y. Clinic, Fukuoka, Japan and who provided written consent to participate in this study. Of these, 6 subjects (2 primiparae, 4 multiparae) agreed to take *Chlorella* tablets (chlorella group). These subjects were asked to take *Chlorella* tablets for approximately 6 months, from gestational week 16~20 up until day of delivery. Dose was 6g/day of *Chlorella* (30 tablets/day), in portions of 10 tablets after each main meal. Biorinck tablets (Chlorella Industry Co. Ltd., Fukuoka, Japan) containing dried *Chlorella* powder as the active ingredient were used. Each tablet comprised the following (g/100g): chlorophyll, 3.2; dietary fiber, 11; protein, 62; lipid, 11. No restrictions were imposed on the remaining participants (1 primipara, 3 multiparae) who comprised the control group, with the exception that they were prohibited from taking *Chlorella* tablets.

This study was conducted in accordance with the general principles of the Helsinki Declaration.

At the beginning of this study, that is, August to December 2010, maternal blood samples (30 ml) were taken from each of the 10 participants and blood concentrations of PCDDs, PCDFs and DL-PCBs in both the chlorella and control groups were determined by high resolution GC/MS method [13], which were expressed as the initial

concentrations.

In order to examine the effects of *Chlorella* on the promotive excretion of PCDDs, PCDFs and DL-PCBs from the maternal body, on the day of delivery, which were between February and April 2011, again maternal blood samples (30 ml) were taken from each of the 10 participants and their blood concentrations were determined by the same analytical method in both the chlorella and control groups, which were expressed as the final concentrations.

Means and standard deviations (SDs) were calculated for the concentrations of each congener, and for PCDDs, PCDFs DL-PCBs and dioxins (PCDDs + PCDFs + DL-PCBs), respectively, for maternal blood. Toxic equivalent (TEQ) concentrations were calculated using WHO toxic equivalency factor values for each congener [14]. In these calculations, measured values of congeners with concentrations below the detection limit were regarded as half of the detection limit.

Statistical significance of the differences between the initial and final concentrations of each group was evaluated by a two-tailed paired student's *t*-test and a *p*-value less than 0.05 was considered significant.

Results and discussion

As shown in Fig. 1, initial mean TEQ concentrations of PCDDs in the maternal blood of the chlorella and control groups were 5.5 and 3.7 pg/g lipid, respectively, and the concentration of chlorella group was about 1.5 times higher than that of control group. However, final mean TEQ concentrations of PCDDs were almost the same in both chlorella and control groups, that is, 3.12 and 3.08 pg/g lipid. Therefore, in chlorella group about 44% reduction was observed, but in contrast only 16% reduction was seen in control group.

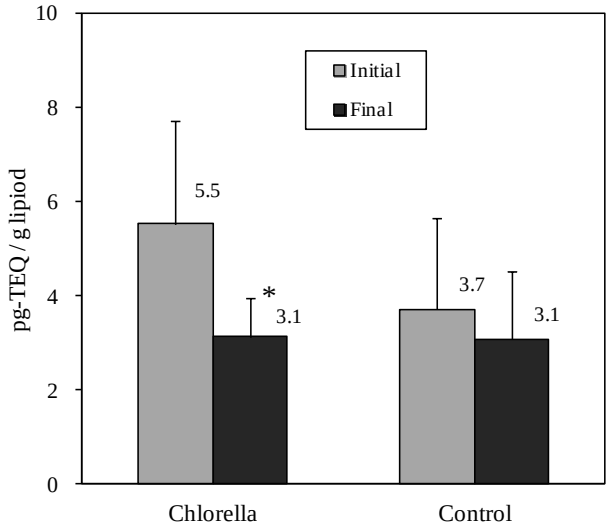

Fig. 1. Mean TEQ concentrations of PCDDs in the maternal blood of chlorella and control groups.
* : p<0.02

In mean TEQ concentrations of PCDFs in the maternal blood, the initial and final concentrations in chlorella group were, respectively, 3.7 and 1.5 pg/g lipid and those in control group 2.7 and 1.5 pg/g lipid. Therefore, reduction rate of chlorella group was 59% and that of control group 44%. Consequently, the reduction rate in chlorella group was much higher than that in control group.

In mean TEQ concentrations of DL-PCBs in the maternal blood, the respective initial concentrations of chlorella and control groups were 3.6 and 1.8 pg/g lipid and the respective final concentrations of both groups 2.5 and 1.7 pg/g lipid.

Hence, the reduction rates of chlorella and control groups were 31% and 5.6%, respectively.
As a consequence, initial and final mean TEQ concentrations of dioxins in the maternal blood of chlorella and control groups, which were indicated in Fig. 2, were 12.8 and 7.2pg/g lipid, and 8.3 and 6.3 pg/g lipid, respectively. Therefore, the reduction rate of chlorella group was 44% and that of control group 24%. Finally, concentrations of dioxins in the maternal blood of chlorella group was 20% more reduced due to the promotive excretion of PCDDs, PCDFs and DL-PCBs from the maternal body probably by *Chlorella* intake than those of control group.
The primary mechanism by which *Chlorella* promotes excretion of dioxins is presumably through the formation of complexes between dioxins and the chlorophyll in *Chlorella* [15]. Dioxins are then absorbed by the dietary fiber also contained in *Chlorella* [16], thereby inhibiting the absorption and reabsorption of dioxins from the gastrointestinal tract.
We have observed very good correlation between the concentrations of dioxins in the blood and those in other tissues such as the liver and adipose tissue [17, 18]. Therefore, the body burdens of dioxins in chlorella and control groups were computed on the assumptions that the mean body weight was 50kg, the body fat was 20% of body weight, that is, 10kg and the body fat was contaminated with the same concentrations of dioxins as those in the maternal blood. Then the initial body burdens of dioxins in chlorella and control groups were, respectively, 128ng and 83ng, and the respective final body burdens of dioxins were 72ng and 63ng. In consequence, the net reduction of body burden in chlorella group was 56ng and that in control group 20ng, as shown in Fig. 3. The respective net reduction rates were 44% and 24%. As already mentioned above, body burden of dioxins in chlorella group was 20% more reduced by *Chlorella* intake than that in control group.
In conclusion, even in this preliminary study, *Chlorella* supplements (Biorinck tablets) seemed very much effective for reducing dioxin exposure, and also considered effective for reducing their risks in fetuses and sucklings.

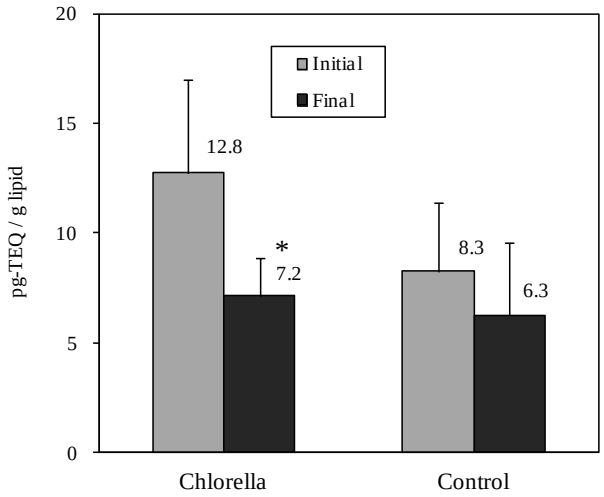

Fig. 2. Mean TEQ concentrations of dioxins in the maternal blood of chlorella and control groups.
* : $p<0.005$

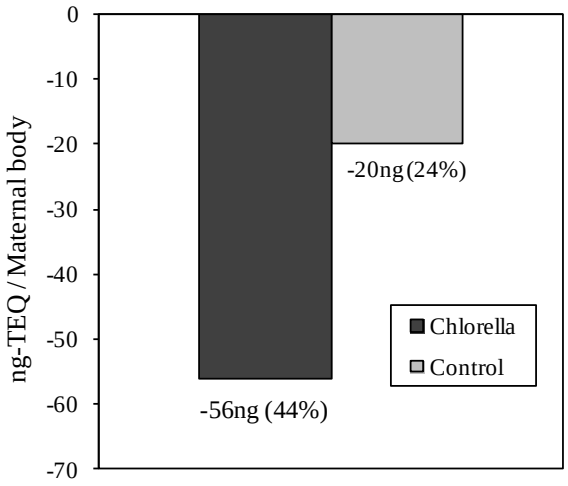

Fig. 3. Promotive reduction of dioxins in body burden by *Chlorella* intake
Figures in parentheses indicate respective reduction rates in chlorella and control groups

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